Remarks

The Telephone Interview

The assignee, Metabolix, and its inventor and representative, Dr. Oliver Peoples, and the undersigned greatly appreciate the helpful telephone interview on March 20, 2007. The interview gave Dr. Peoples an opportunity to explain how the company was using several different recombinant bacterial strains to produce different polyhydroxyalkanoates, and that the discoveries claimed herein represented a major factor in being able to produce these biodegradable plastics on a large scale and commercially feasible basis. In this industry, it is critical to decrease cost to a very low level in order to be competitive with the petroleum derived plastics. The development of the bacterial strains which produce the polyhydroxyalkanoates to a high level in large industrial fermenters and can express adequate nuclease to reduce the viscosity and enable cost effective recovery of the polyhydroxyalkanoate took many years. The significance of this development has been independently recognized by others in the field having no economic or other relationship to Metabolix, as demonstrated by the enclosed review by Weusthuis, et al., in BIOPOLYMERS, eds. Steinbuechel, et al., pp 291-317, WILEY-VCH Verlag GMBH, Rep of Germany, 2002 (see especially page 306, the paragraph spanning the left and right columns).

As discussed in the telephone interview, Dr. Peoples can submit a Declaration under 37 C.F.R. 1.132 attesting to the long felt need for a solution to cost effective large scale production of polyhydroxyalkanoates, the many years and high cost of development of the claimed technology, the development of multiple bacterial hosts for production of a variety of

polyhydroxyalkanoates, and the recognition by others in the field, all strong indicia of non-obviousness, should this be helpful.

Amendments to the Claims

Claim 1 has been amended to incorporate the limitations of claim 3. Support for this amendment can . No new matter is introduced.

Claim 7 has been rewritten in independent form.

All claims have been cancelled or amended to restrict the fermentation product to polyhydroxyalkanoates; the nuclease to an integrated heterologous nuclease, and the culture to a high density culture. Antecedent basis has been corrected as necessary.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-10 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection if applied to the amended claims.

The Legal Standard

The general standard for the written description requirement is that "a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention." *See* M.P.E.P. § 2163(I). All that is required is that the specification provides sufficient description to **reasonably** convey to those skilled in the art that, as of the filing date sought, the inventor was in possession of the claimed invention. *Union Oil of California v. Atlantic Richfield Co.* , 208 F.3d 989, 997, 54

U.S.P.Q.2d 1227, 1232 (Fed. Cir. 2000); *Vas Cath*, 935 F.2d at 1563-64. An applicant may show possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997). As noted in a recent decision by the Board of Appeals and Interferences, the written description requirement does not require a description of the complete structure of every species within a chemical genus. (*see Utter v. Hiraga*, 845 F.2d 993, 998, 6 U.S.P.Q.2d 1709, 1714 (Fed. Cir. 1988), stating "A specification may, within the meaning of 35 U.S.C. § 112, para. 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses.").

An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention. *Id.*, citing *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000); *Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 3 11, 48 USPQ2d at 1646 (1998).

In a decision by the Board of Patent Appeals and Interferences, the Board warned that it is an improper analysis to determine that the claims are directed to an invention which is broader than that which is described in the specification since the <u>written description is determined from the perspective of what the specification conveys to one skilled in the art</u> citing *In re GPAC Inc.*, 57 F.3d 1573, 1579. 35 USPQ2d 1116, 1121 (Fed. Cir. 1995) and *Vas Cath*, 935 F.2d at 1563-64. Thus the Board re-emphasized that the specification need not always spell out every detail;

only enough "to convince a person of skill in the art that the inventor possessed the invention and to enable such a person to make and use the invention without undue experimentation." *LizardTech Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1344-34, 76 USPQ2d 1724, 1732 (Fed. Cir. 2005).

Although the "written description" requirement is a separate requirement from the "enablement" requirement, **if the enablement requirement has been met, it is difficult for the Examiner to assert that the written description requirement has not similarly been met**. The Federal Circuit recently expressed this in *LizardTech Inc. v. Earth Resource Mapping, Inc.*, stating "A recitation of how to make and use an invention across the full breadth of the claim is ordinarily sufficient to demonstration that the inventor possesses the full scope of the invention and vice versa." *LizardTech Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336, 1343, 76 U.S.P.Q.2d 1724. 1732 (Fed. Cir. 2005).

Analysis

The claims define a bacterial strain for producing a polyhydroxyalkanoate, wherein the bacterial strain is genetically engineered to integrate a heterologous nuclease which is expressed and the nuclease is secreted in an amount effective to degrade at least 95% of a nucleic acid following cell lysis in less than 24 hours, thereby reducing viscosity, thereby enhancing product recovery. Using bacterial strains, nucleases and genetic engineering techniques which are known in the art (as discussed below), the present Application describes how to produce novel organisms that secret the desired amount of nuclease. The claimed organisms which would be

beneficial in decreasing the overall fermentation process costs by increasing the recovery of the product from the cell biomass and in some cases, from the medium.

Bacterial strains, such as *Ralstonia*, *Aeromonas*, *Azotobacter*, *Burkholderia*, *Comamonas*, *Methylobacterium*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, and *Zooglea*, have been sold by the American Type Culture Collection in Rockville, MD and used in school laboratories and commercial fermentation facilities for many years. All are well known to be amenable to typical manipulations of bacterial genetics, allowing the use of broad host range cloning vectors as transforming vehicles for a nuclease gene of interest (see at least the paragraph bridging pages 7 and 8).

Suitable nuclease genes were well known and described in the literature with specific sources taught in the specification at least at page 6, lines 4-13, and can be obtained and produced by using well established methods in the art, such as PCR and primers complementary to the sequence encoding the nuclease using information obtained from publicly available databases. Examples of such sequences are disclosed for many strains in GenBank (see at least page 6, lines 4-13; and page 7, lines 15-22). Once the nuclease gene has been isolated, common genetic manipulation allows for its integration into a microbial strain (see at least page 7, lines 8-10).

The present application describes how to isolate a nuclease of interest and integrate the gene into bacteria of interest. These strains can be screened for the desired characteristics with no further information than a simple assay for nuclease activity, or the desired viscosity of the cell lysate. Neither requires more than routine experimentation. Example 1, describes isolation

of a suitable nuclease gene (page 11, line 30 to page 12, line 20); Example 2, construction of a vector to insert the nuclease gene into a *P. putida* bacteria (page 12, line 21 to page 13, line 16), screening for nuclease expressing clones (12,000 random integrants; 1500 colonies screened; 35 nuclease expressing clones; 9 secreting nuclease); Example 3, screening of *R. eutropha* bacterial strains for secretion of nuclease (1/10 produced nuclease in the periplasm) (page 14, lines 16-23). Table 1 on page 15 shows the amount of nuclease secreted into the periplasm for six strains, of which three are high producers and one very high (MBX 979). Example 6, demonstrates the actual isolation of products from cell lysates from an engineered, screened bacterial strain, MBX 985, and a non-engineered strain (page 16, line 19 to page 17, line 5).

The examiner has failed to provide any evidence or reasoning as to why those skilled in the art would not extrapolate from the actual examples in the application to other strains of bacteria or other nuclease genes (see the specification at least at page 6, lines 9-10) and screen for strains expressing desired levels of nuclease as Applicants have done for heterologous expression of the *Staphylococcus aureous* nucleas in *P. Putida*. Nuclease activity assays, PCR isolation of nuclease genes from chromosomal DNA, PCR isolation of nuclease genes from DNA utilizing knowledge obtained from sequences already disclosed as GenBank reference numbers, cell lysis methods to render accessible product and nuclease (if periplasmically localized), and, in general, what is already known about product recovery from bacterial strains, are all methods and relevant subject matter taught in the present specification and readily realized by one of ordinary skill in the art as commonplace in the field. Applicants respectfully

submit that in view of these disclosed methods and what is already known, one of skill would have no problem isolating nuclease genes and transforming a fermenting bacterial host strain.

No rationale has been presented for why the specification fails to provide sufficient written description for strains wherein the nuclease gene is integrated into a host strain selected from the group consisting of *Ralstonia eutropha, Methylobacterium organophilum, Methylobacterium extorquens, Aeromonas caviae, Azotobacter vinelandii, Alcaligenes latus, Pseudomonas oleovorans, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas aeruginosa, Pseudomonas acidophila, Pseudomonas resinovorans, Escherichia coli, and Klebsiella* (claim 7).

The specification and examples provide clear support for the entire breadth of the claimed subject matter. Therefore, Claims 1, 4, and 7 meet the written description requirement.

Rejection Under 35 U.S.C. § 102

Claims 1, 2, 4, 5, 6 and 8 were rejected under 35 U.S.C. § 102(b) as anticipated by Liebl, et al., *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"). Applicants respectfully traverse this rejection if applied to the amended claims

The Legal Standard

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc. v Monoclonal Antibodies Inc.*, 231 USPQ 81 (Fed. Cir. 1986), cert. denied, 480 US 947 (1987); *Scripps Clinic & Research Found. v Genentech Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 USPQ2d at 1010:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. (Emphasis added)

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps*, Id.:

> [A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts beyond those disclosed in the reference in order to meet the claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

For a prior art reference to anticipate a claim, it must enable a person skilled in the art to make and use the invention. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled". *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 USPQ2d 1385, 1416 (Fed. Cir. 2003).

Analysis

The claims require the heterologous gene encoding the nuclease be integrated.

Liebl does not disclose an integrated gene, but a plasmid that requires induction for expression. Liebl teaches Staphylococcal nuclease (SNase) expression by various *C. glutamicum*

strains, wherein the *C. glutamicum* transgenic strain is to be used for investigating protein export and processing. Liebl is concerned with investigating protein secretion in *C. glutamicum*.

Liebl does not disclose a bacterial strain for the production of a fermentation product which is genetically modified to express a chromosomally integrated heterologous nuclease gene. Furthermore, Liebl does not disclose a bacterial strain for the production of a fermentation product which is genetically modified to express a chromosomally integrated heterologous nuclease gene wherein the nuclease gene product is secreted into the periplasmic space or culture medium in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of the cells in less that 24 hours to reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of the product is enhanced. The present application shows in Example 6, that Applicants' genetically modified bacteria secrets nuclease into the culture medium in one hour, in amounts that reduce viscosity of the culture medium to levels that are equivalent to those achieved by commercial Benzonase. For a reference to anticipate the claims, it must recite each and every limitation of the claims. A reference which fails to disclose any one of the claim limitations cannot anticipate the claims.

Liebl does not disclose a bacterial strain containing at least 40% polyhydroxyalkanoate by dry cell weight. Liebl does not disclose or suggest the <u>reduction of viscosity of a cell lysate</u> as a result of secreting a nuclease into the periplasm or growth medium. Liebl does not disclose or suggest what an effective amount of the secreted nuclease would be, that would reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50g/l so that the recovery of the product is enhanced. The Examples in the present application clearly show that a

high level of nuclease expression in the constructed strains is required to generate commercial levels of product (for example polyhydroxyalkanoate). For example, the claims are directed to fermenting bacterial strains capable of growth to cell densities of at least 50g/l and producing polyhydroxyalkanoates to levels of at least 40% of dry cell weight. Liebl does not disclose a bacterial strain capable of growing to densities of at least 50g/L as asserted by the Examiner. Liebl shows optical densities of approximately OD600=5.0 (Liebl, Figure 2). A person of ordinary skill in the art would be aware that in industrial biotechnology, a reasonable estimate of cell density is gotten by dividing the OD600 value by 3 or 4. Using this calculation for the optical densities observed in Liebl, that would imply that the cells grow to densities of about 1.25-1.66 g/L. The Examples illustrate fermenting bacterial cell cultures grown to a cell density of 200 g/l in 20 L fed-batch cultures, clearly indicating the need for high level nuclease expression in order to sufficiently reduce lysate viscosity and enhance product recovery (see Example 6). The high level of expression of the nuclease is reflected in Table 2, wherein the viscosity of each batch lysate is comparable to wild type cultures supplemented with BENZONASE as shown in Example 6.

However, the Examiner asserted that a teaching of enhancement of product recovery as a direct result of secreting a nuclease into the periplasm or growth medium by the bacterial strain is not a limitation of the anticipated claims. The claims as amended, recite that the nuclease gene product secreted into the periplasmic space is an amount effective to degrade at least 95% of all nucleic acid released following lysis in less than 24 hours, to reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50g/l so that recovery of the product is

enhanced. Even if the claims recited such a limitation, the Examiner asserted that C. glutamicum R163/pWLQN10 inherently expresses the nuclease in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less than 24 hours, since *C. glutamicum* R/163pWLQN10 produced approximately 80- fold higher SNase activity after induction as a result of IPTG supplementation of the medium (approximately 40 units/ml after 24 hours in culture). Applicants respectfully disagree. There is no teaching or suggestion in Liebl of degradation of nucleic acids following lysis, or what amount of nuclease would be effective in degrading 95% of the nuclease released. In relying upon the theory of inherency, the Examiner **must** provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. The Examiner has not done so in this instance. According to the MPEP §2112(IV), "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' " *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999). Even if the Examiner's assertion were correct that the nuclease be effective in degrading 95% of all nucleic released following cell lysis, the Examiner's attention is drawn to the fact that 40 Units/ml of SNase is secreted following IPTG

supplementation of the culture medium in Liebl. Genetic modification involves the isolation, manipulation and reintroduction of DNA into cells or model organisms, usually to express a protein (see http://en.wikepedia.org/wiki/Genetic_engineering, a copy of which is attached). The Examiner attention is drawn to Fig 2B, which shows the secretion of SNase in culture medium by *C glutamicum* R163/pWLQN10, without the addition of IPTG in the culture medium. This strain of bacteria is an example of a strain overexpressing the SNase gene (see Liebl, results section entitled "overexpression of SNase in *C. glutamicum*). Overexpression of the nuclease by putting it under the control of a stronger promoter in R163/pWLQN10 strain (Fig 2B) showed enzyme activities of about 0.1U/ml at 2.5 hours and about 0.5 Units/ml in 24 hours (without IPTG treatment). **This is approximately the amount of enzyme Liebl's "genetic modification" was able to produce.** IPTG treatment is not genetic modification. Furthermore, even if IPTG treatment could be characterized as genetic modification as the Examiner has done, IPTG as an inducing agent at large scale would be cost prohibitive. On the contrary, the present application shows in Example 6, that Applicants' genetically modified bacteria secrets nuclease into the culture medium in one hour, in amounts that reduce viscosity of the culture medium to levels that are equivalent to that reduced by commercial Benzonase (which are sold with activities of 250U/µl, see http://www.biocompare.com/matrix/13891/Benzonase.html). If one were to extrapolate as the examiner has done, it would be clear that in 24 hrs, the enzyme activity in the culture medium of the claimed bacteria strain would be superior to that seen in Liebl. The Examiner has repeatedly cited to the 80-fold increase in the amount of nuclease secreted R163/pWLQN10 in Liebl upon treatment of the medium with IPTG, as an inherent anticipation

of "levels effective to degrade 95% secreted nucleic acid". It is clear that the Examiner recognizes that the genetically modified bacteria in Liebl does not secret appreciable levels of nuclease, **until** chemical treatment with IPTG. Furthermore, the nuclease gene disclosed in Liebl is not integrated into the chromosome of the engineered bacterial strain as required by the claims. The bacteria disclosed in Liebl is different from the claimed bacterial strain.

The Examiner also asserted that a single cleavage event of a nucleic acid molecule could be considered to be encompassed by "degrade" as recited in the claims, and thus the bacterial strain taught by Liebl secretes the expressed nuclease in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less than 24 hours. Applicants are not clear as to the Examiner's point here, however, the claims require that the mount of nuclease secreted degrade 95% of all of the nucleic acid released and decrease viscosity (determined for example with a Viscometer; see Example 16 of the present specification. It is apparent that a single cleavage event would not result in the degradation of 95% of all of the nucleic acids released.

As previously stated, Liebl does not show that expression decreases viscosity. Liebl does not show that a decrease in viscosity is inherent or that it is done at an appropriate level to decrease viscosity. No product is made by Liebl. Liebl expresses nuclease only in the absence of a product such as PHA. The presence of product would certainly increase the viscosity because there is more material present. Liebl is not the same system as the present application, nor is it trying to achieve the same result. The presence of the product makes the present system different from Liebl. Liebl does not enable one of ordinary skill in the art to obtain a bacterial

strain for production of a fermentation product by expressing a nuclease in an effective amount to reduce viscosity in order to obtain the fermentation product. Nothing in Liebl allows one of ordinary skill in the art to say that the enzyme's activity will decrease the viscosity. Therefore, claims 1, 2, 4, 5, 6 and 8 are not anticipated by Liebl.

Rejection Under 35 U.S.C. § 103

Claims 1-10 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 by Greer, et al., ("Greer"), Atkinson, et al., Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, et al., *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), or Miller, et al., *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller") in view of Liebl or Miller. Applicants respectfully traverse this rejection.

The Legal Standard

The U.S. Patent and Trademark Office has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Warner et al.*, 379 F.2d 1011, 154 U.S.P.Q. 173, 177 (C.C.P.A. 1967), *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598-99 (Fed. Cir. 1988). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of

success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The prior art must provide one of ordinary skill in the art with the motivation to make the proposed modifications needed to arrive at the claimed invention. *In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987); *In re Lalu and Foulletier*, 747 F.2d 703, 705, 223 U.S.P.Q. 1257, 1258 (Fed. Cir. 1984). Claims for an invention are not *prima facie* obvious if the primary references do not suggest all elements of the claimed invention and the prior art does not suggest the modifications that would bring the primary references into conformity with the application claims. *In re Fritch*, 23 U.S.P.Q.2d. 1780 (Fed. Cir. 1992). *In re Laskowski*, 871 F.2d 115 (Fed. Cir. 1989). This is not possible when the claimed invention achieves more than what any or all of the prior art references allegedly suggest, expressly or by reasonable implication.

The case law has **clearly** established that the cited references **must** recite each and every element of the claims **as well as** provide to one of skill in the art the motivation to combine the cited references **and** provide one of ordinary skill in the art with a reasonable expectation of success. The references cited by the Examiner clearly do not satisfy these criteria.

Analysis

Greer

Greer describes the exogenous addition of peroxide to a cell culture. As stated in the Examples of Greer, and as stated as one of the problems addressed by the presently claimed invention, the exogenous addition of nucleases is generally known and too expensive to use for

commodity fermentation products involving high cell density fermentations. Applicants are using elevated expression of nuclease instead of peroxide addition.

Liebl

Liebl describes the heterologous expression of a *Staphylococcus aureus* nuclease gene in *C. glutamicum* and the use of this transgenic system for investigating protein export in *C. glutamicum*, as discussed above. However, there is no disclosure of possible uses for the disclosed nuclease, other than for elucidating protein export and processing mechanisms. Liebl does not disclose how to engineer then screen for very high levels of secreted nuclease activity which according to the3 Examples, are necessary to degrade 95% of nucleic acid secreted, in order to enhance product recovery. Liebl does not disclose a bacterial strain that contains at least 40% polyhrdoxyalkanoate by dry cell weight. One would therefore not arrive at the claimed strain from the disclosure in Liebl. None of the secondary references make up for the deficiencies in Liebl. Miller

Miller teaches the use of a *B. subtilis* secreted nuclease for investigating "the nature of the processing of the nuclease signal peptide". Miller further characterizes the secretion of nuclease and the processing of the signal peptide from the precursor protein in *B. subtilis*. Miller speculates that the *staphylococcal* nuclease and its gene may be very useful for the development of secretion vectors for foreign proteins. There is no teaching in Miller to select for strains which secrete into the periplasm or growth medium so that recovery of a large scale product is enhanced. As discussed in reference to Liebl, the disclosure in Miller similarly does not enable one of ordinary skill in the art to arrive at the claimed bacterial strains.

Atkinson

Atkinson is a general review of biochemical and biotechnological methods and reagents.

Lee

Lee reports on production of PHAs in bacteria, and control of fermentation conditions.

The References in Combination

(a) The Prior Art Fails to Disclose or Suggest Each and Every Element of the Claims

The claims require a bacterial strain that can produce both a fermentation product and secret a nuclease in amounts effective to degrade 95% of nucleic acid in the growth medium upon cell lysis, and reduce viscosity of the cell lysate to enhance recovery of product. The claims also require that the heterologous nuclease gene be intergrated into the chromosome of the bacterial strain, and that the bacterial strain contain at least 40% PHA by dry cell weight. None of the prior art references either alone or in combination discloses or suggest a genetically engineered bacterial strain that possesses these attributes. Therefore, the prior art references, either alone or in combination, failed to teach or suggest each and every element of the claims as required under 35 U.S.C. § 103. *In re Fritch* 972 F.2d 1260, 23 USPQ2d 1780 (Fed. Cir. 1992); *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

(b) The Prior Art Does not Provide Motivation to Combine

Contrary to the examiner's assertion, Greer, Atkinson, and Lee or Miller in view of Liebl or Miller do not provide a motivation for one of ordinary skill in the art to express a nuclease by genetic engineering to degrade nucleic acid in a growth medium for the enhanced recovery of a fermentation product. Liebl and Miller heterologously express nucleases in Bacteria. Greer

states that purified preparation of nucleases are expensive. The Examiner has repeatedly used this statement in Greer as a motivation to genetically engineer bacteria to secret a nuclease. Greer is simply stating a problem and in no way proposing a solution that requires genetic engineering. In fact, Greer proposes a precipitating agent such as polyethylene as a significantly cheaper alternative to nucleases (see Greer, page 1, lines 29-31). It is unclear how the statement that nucleases are expensive would motivate one of ordinary skill in the art to genetically modify bacteria to express high levels of nuclease as Applicants have done, without any disclosure or suggestion as to the benefits of such a modification. None of Liebl or Miller discloses a bacterial strain engineered to secrete nuclease effective to degrade 95% of nucleic acid released following cell lysis. According to the Examiner, the motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which results in an increase in the medium, causing problems in the downstream processing steps, as taught by Greer. Greer does not disclose or suggest genetic engineering as a solution to this problem. Greer suggests using a precipitating agent. Neither Miller nor Liebl are concerned with reducing viscosity in a fermentation process. None of Miller or Liebl discloses or suggests what the effective amount of nuclease secreted would be, that would degrade 95% of nucleic acid released if the cells grown at a density of 50g/L are lysed and therefore enhance product recovery. The issue is not simply that bacteria be able to secret nuclease into the culture medium. The amount has to be effective in degrading 95% of the nucleic acid secreted upon cell lysis of a cell lysate in a cell culture having a density of 50 g/l to reduce viscosity and enhance product recovery. There is no motivation to combine these

references with Greer as the Examiner has done. As stated under the argument for anticipation regarding Liebl, Liebl does not disclose a bacterial strain engineered such that the heterologoulsy expressed gene is integrated into the host chromosome, enabling the bacteria strain to secret nuclease in an amount to degrade at least 95% of nuclsic acid following cell lysis, without the addition of any chemical inducing agents. Liebl does not disclose the claimed bacteria. According the MPEP §2142 "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)." It is clear that the Examiner is using hindsight reconstruction that had been repeatedly discredited by the courts. *See i.e.*, *In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999); *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

(c) The Prior Art Does not Provide a Reasonable Expectation of Success

The prior art can not lead one of ordinary skill in the art to have a reasonable expectation of success. The Examiner has cited to Miller and Liebl as the reference that provide an expectation of success, since Miller and Liebl show that it is possible to heterologously express a nuclease in bacteria. Liebl does not show how to heterologously express a nuclease in bacteria such that the nuclease gene integrates into the bacteria chromosome. The issue here in not whether a nuclease can be heterologously expressed in bacteria. The issue is to express the gene such that the amount of enzyme secreted is effective in degrading 95% of nucleic acid secreted. A teaching of successful heterologous expression of a nuclease gene in bacteria (to study protein

secretion) using plasmid expression systems that require induction is not sufficient to make obvious the present claims.

In order for one of ordinary skill in the art to successfully make or use the claimed subject matter, there are at least two hurdles to overcome: (1) the co-expressing of the enzymes that produce the product (PHA) as required in the claims and the nuclease that degrades nucleic acids in the growth medium for the product, wherein the nuclease gene integrates into the chromosome of the bacteria, and (2) the expression of the nuclease in an effective amount for the reduction of viscosity of the growth medium. As the foregoing discussion demonstrates, the prior art references, individually or in combination, fail to teach or suggest the co-expression of the nuclease and the enzymes that produce the product listed in the claims. Furthermore, nowhere is there any teaching that would lead one to combine a heterologous gene encoding a nuclease which is expressed and then secreted into either the periplasmic space or the growth medium in an amount effective to degrade nucleic acid sufficient to decrease viscosity and more economically obtain a product produced by bacterial fermentation.

The examples further demonstrate that one of ordinary skill in the art can not have a reasonable expectation of success of the claimed subject matter. *See* Examples 1-6. Indeed, just looking at the examples one realizes that most of the samples that were screened did not integrate the nuclease gene, did not secrete nuclease either into the cell culture medium or periplasmic space, and did not produce sufficient quantities of nuclease to reduce the viscosity of the cell culture medium and would therefore not have been useful. Therefore, one of ordinary skill in the art can not have a reasonable expectation of the success of the claimed subject matter.

The cited art either individually or in combination do not disclose a bacterial strain for the production of PHA, wherein the bacterial strain is genetically modified to express a heterologous nuclease gene by integration of the nuclease gene, wherein the nuclease gene product is secreted into the periplasmic space or culture medium in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of the cells in less than 24 hours to reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of the product is enhanced, which produces a polyhydroxyalkanoate to levels of at least 40% of its dry cell weight.

Accordingly, claims 1, 4 and 7 are not obvious over Greer, Aktinson, and Lee or Miller in view of Liebl or Miller.

Allowance of claims 1, 4 and 7, and rejoinder and allowance of claims 11, 12 and 14-16 is respectfully solicited. Claims 11, 12 and 14-16 are related to claims 1, 4 and 7 as product and process of use. Accordingly, no new search would be required should claims 1, 4 and 7 be found to be allowable.

Respectfully submitted,

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